Effects of Dietary Supplementation with *Yucca schidigera* Roezl ex Ortgies and Its Saponin and Non-saponin Fractions on Rat Metabolism

Cepta F. Duffy,*,† Gerry F. Killeen,‡ Cathal D. Connolly,† and Ronan F. Power†

Alltech European Biosciences Research Center, The National University of Ireland, Galway, Ireland

Yucca schidigera Roezl ex Ortgies, family Lillaceae, was fractionated with butan-1-ol to yield a butanol extractable fraction (BE; saponin fraction) and a non-butanol fraction (NBE; non-saponin fraction). Four groups of eight male rats were allowed ad libitum access to diets supplemented with water (control) or 200 mg kg^{-1} total *Y. schidigera* (TOT) or 200 mg kg^{-1} of each of the fractions (NBE or BE). The effects of dietary supplementation with the fractions and their interactions in TOT were analyzed according to the factorial experimental design by two-way analysis of variance. All three supplementation groups displayed significantly reduced serum urea levels ($P < 0.05$). The TOT and NBE fractions were found to significantly increase serum insulin levels ($P < 0.01$) in the absence of any fluctuations in serum glucose levels. Urea cycle enzyme activities, namely, arginase (EC 3.5.3.1) and argininosuccinate lyase (EC 4.3.2.1), were significantly decreased ($P < 0.05$) in vivo, although no effect was observed in vitro. Both fractions displayed effects, indicating that the active constituents are present in both fractions.

Keywords: *Yucca schidigera; saponin; animal feedstuff; supplementation*

INTRODUCTION

Extracts and preparations of the desert plant *Yucca schidigera* Roezl ex Ortgies (Mohave yucca), family Lillaceae, have a variety of beneficial effects when included in the diets of humans and domestic animals. Preparations have been approved by the U.S. Food and Drug administration as an additive for human consumption (*1*) and are commonly used as flavoring or foaming agents.

Since *Y. schidigera* extract has become commercially available, a number of investigations into its effects on a wide range of animals have been carried out. These studies have shown that the extract has many positive benefits on growth and performance (*2*−*4*), health (*5*−*7*), and reduced gastrointestinal and fecal ammonia levels (*8*−*10*). Although an abundance of data already exists on the effects of dietary *Y. schidigera* supplementation, the mechanism of action remains unresolved. Several mechanisms have been proposed, but none have been conclusively proven. Until recently *Y. schidigera* extract was thought to be a potent in vitro inhibitor of the enzyme urease (urea amidohydrolase, EC 3.5.1.3) (*9*, *11*) and its in vivo properties were attributed to inhibition of gastrointestinal urease (*4*, *6*, *12*).

However, other studies have questioned the methodologies used to measure the inhibition of urease in a number of these cases, indicating that any observed inhibition of enzyme activity may have been artifactual (*13*, *14*). Thus, it is possible that direct inhibition of gastrointestinal urease by *Y. schidigera* extract may not be a tenable explanation for its mode of action.

Other proposed mechanisms of action include stimulation of microfloral growth (*15*), direct binding of ammonia (*10*), and inhibition of selected gut microorganisms (*16*), in particular, rumen protozoa (*17*). *Y. schidigera* has been shown to influence microbial processes in a variety of fermentation systems in vitro (*15*, *18*, *19*) and in vivo (*9*, *14*, *20*), which has led to an increasingly popular belief that its mode of action is directly related to modulation of microbial populations in vivo. Regardless of the mode of action of the extract, however, it is interesting to note that the biological activity of *Y. schidigera* has historically been attributed to its constituent sapogenins. These are triterpenoidal or steroidal compounds that are found primarily but not exclusively in plants (*21*). Sarsapogenin and smilagenin are the predominant forms of these compounds found in *Y. schidigera* (*22*), and it has been previously demonstrated that it is possible to extract >98% of the total sarsapogenin and smilagenin contents of *Y. schidigera* with butan-1-ol (*23*, *24*).

In an attempt to clarify the role of the saponin fraction of *Y. schidigera*, a trial was conducted using laboratory rats to investigate the effects of dietary supplementation with *Y. schidigera* extract or its constituent butanol extractable fraction (BE; saponin fraction) or non-butanol extractable fraction (NBE; non-saponin fraction), respectively, on a number of variables, namely, serum, excretory, and hepatic variables, with particular emphasis on measurements relating to overall nitrogen metabolism. The main objective of the experiment was to determine if the biological effects of *Y. schidigera* supplementation were due exclusively to its saponin

* Address correspondence to this author at the Alltech Biotechnology Center, Sarney, Summerhill Road, Dunboyne, Co. Meath, Ireland (telephone +353 1 8262244; fax +353 1 8262244; e-mail cduffy@alltech-bio.com).

† Present address: Alltech Biotechnology Center, Sarney, Summerhill Road, Dunboyne, Co. Meath, Ireland.

‡ Present address: Mbita Point Research and Training Center, International Center for Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya.

10.1021/jf010149+ CCC: $20.00
Published on Web 06/07/2001

Table 1. Composition of Standard Pelleted Laboratory Rat Diet[a]

constituent	content	constituent	content
crude protein (g kg^{-1})	170.0	vitamin A (IU kg^{-1})	9000
crude fiber (g kg^{-1})	25.0	vitamin D_3 (IU kg^{-1})	2000
crude oil (g kg^{-1})	75.0	vitamin E (IU kg^{-1})	60
crude ash (g kg^{-1})	75.0	copper (mg kg^{-1})	15
moisture (g kg^{-1})	150.0		

[a] Constituents in descending order of inclusion (manufactures specifications): cereal grains, cereal grain product and byproducts, products and byproducts of sugar production, oil seed products and byproducts, fish products, dried forages, minerals. Note that the diet contained no antibiotic.

constituents, other non-saponin compounds, or, alternatively, an interactive effect between both fractions.

EXPERIMENTAL PROCEDURES

Materials. Unless stated otherwise all reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Aqueous *Y. schidigera* extract [22.6% (w/v) *Y. schidigera* solids] was obtained from Alltech Inc. (Nicholasville, KY). The concentrate was extracted into butan-1-ol to yield a butanol extractable fraction (BE) and a non-butanol extractable fraction (NBE) as described previously (*14*).

Animals, Diet, and Housing. Thirty-two male Wistar (WU strain) rats were obtained at an average age of 3 weeks. The animals were maintained for 2 weeks before the experiment commenced by allowing ad libitum access to water and standard pelleted laboratory diet (Table 1) obtained from W. Connolly and Sons Ltd., (Red Mills, Goresbridge, Co. Kilkenny, Ireland).

For supplementation of standard pelleted diet (Table 1), aliquots (400 μL) of BE or NBE were mixed with 100 mL of water in the reservoir of a glass aerosol spray unit (Fisons, Sussex, U.K.) and sprayed onto 2 kg of the standard diet to obtain as even a coating as possible. Another 100 mL of water was added to the reservoir and the process repeated to ensure complete transfer of the extract. The total *Y. schidigera* (TOT)-supplemented group was administered an equal volume (400 μL) of both BE and NBE and recombined in the sprayer reservoir before spraying onto 2 kg of the standard diet as before. This equates to a supplementation level of 200 ppm for BE, NBE, and the recombined TOT. The control feed was also subjected to spraying with 200 mL of water onto 2 kg of feed.

The 32 animals were tagged by ear punch and were randomly assigned to eight cages, forming four groups of eight that did not differ ($P > 0.4$) with respect to the means or variances of their weights. The animals were then allowed ad libitum access to water and basal diet supplemented as follows: with water (control), BE, NBE, or TOT as described above. The control and *Y. schidigera*-supplemented groups had a two-way design as the TOT treatment was equivalent to a combined treatment with both BE and NBE. The animals remained healthy throughout the the experiment.

Sample Collection, Preparation, and Preservation. On the last days of the experiment the animals were weighed and euthanized by decapitation. Four rats from each group were killed on each of days 75 and 76 to allow rapid assay of serum ammonia and to restrict liver harvesting to the mornings because activities of urea cycle enzymes are known to fluctuate diurnally (*25*, *26*). After decapitation, blood was collected in an ice-cold beaker, allowed to clot for 15 min, and then centrifuged at 1000*g* for 15 min. The serum was subsequently removed and stored at −70 °C. Liver samples were removed, wrapped in foil, immediately frozen in liquid nitrogen, and stored at −70 °C. Liver homogenates used for assaying urea cycle enzyme activities and protein content were homogenized in a standard manner for 60 s in 10 mL of ice-cold water using a mechanical Potter homogenizer.

Freshly excreted urine samples were collected at the time of weighing and frozen immediately for subsequent determination of urea and ammonia.

The hepatic samples used for urea cycle enzyme analysis in vitro were obtained from a male Sprague−Dawley rat. After decapitation, the complete liver was rapidly harvested, wrapped in foil, and immediately frozen in liquid nitrogen before storage at −70 °C. Liver was homogenized in 10 mL of ice-cold water in a standard manner using a mechanical Potter homogenizer for 60 s. This homogenate was used for all in vitro urea cycle enzyme activity assays.

Analytical Methods. *Ammonia, Urea, and Urease.* Serum ammonia was determined according to a modification of the method of Mondzac (*27*) using a kit following the manufacturer's (Sigma) instructions. In all other cases, ammonia and urea (measured as ammonia, its degradation product) were determined according to a modified Berthelot method based on the method of Chaney and Marbach (*28*), as described by Killeen et al. (*13*).

Creatinine, Glucose, and Insulin. Serum creatinine was determined using a kit according to the manufacturer's (Sigma) instructions.

Serum glucose was determined using a kit according to the manufacturer's (Sigma) instructions.

Serum insulin concentrations were determined by radioimmunoassay (RIA) (*29*).

Urea Cycle Enzyme Activities. Liver urea cycle enzyme activities were determined from a liver homogenate (10% w/v) prepared in H_2O. Urea cycle enzymes arginase (ARG; EC 3.5.3.1), argininosuccinate lyase (ASL; EC 4.3.2.1), carbamoyl phosphate synthase (CPS; EC 6.3.4.16), and ornithine transcarbamolyase (OTC; EC 2.1.3.3) were assayed according to the method of Schimke (*30*), as modified by Nazum and Snodgrass (*31*). The unit of activity of the urea cycle enzymes is expressed as micromoles of product formed per minute per gram of liver at 37 °C.

Statistical Methods. Where the variance of the untransformed data was found to be homogeneous among the groups by all possible pairwise *F* tests, two-way analysis of variance (ANOVA) (*32*, *33*) was applied according to the treatment structure. This was accomplished by treating the TOT as the combination of its two fractions, BE and NBE.

Serum insulin concentrations were log-transformed for statistical analysis by two-way ANOVA as appropriate for such log-transformed statistical data (*32*, *34*). The results of this log-normal distributed variable are presented as back-transformed means and a pooled 95% confidence interval (CI) of the log-transformed data.

Statistical calculations were carried out using a PC version of Minitab (Minitab Inc., State College, PA, 1988) and Fig. P (Biosoft, Cambridge, U.K., 1992).

RESULTS

Effects on Serum Variables. Serum urea levels were significantly reduced by *Y. schidigera* supplementation in all three treatment groups, TOT, NBE, and BE, by 21, 15, and 16%, respectively (Table 2). In contrast to the observed decreases in serum urea concentration, total *Y. schidigera* extract and both of its constituent fractions had no effect on serum ammonia concentrations (Table 2). Although it is noteworthy that a decrease of up to 36% in serum ammonia concentrations relative to controls was observed in the groups supplemented with both the NBE and BE fractions, these appear to cancel each other out in the recombined TOT-supplemented group.

Serum insulin levels were significantly increased in TOT and NBE treatment groups (Table 2) by 195 and 135%, respectively. The fact that serum glucose concentrations were not elevated (Table 2) indicates that the increase in serum insulin concentration is not a response to an increase in serum glucose levels.

Table 2. Influence of Feed Supplementation with *Y. schidigera* Extract and Its NBE and BE Fractions on Serum Variables[a]

variable	control	*Y. schidigera*			SEM	effect[b]	P^b
		NBE	BE	TOT			
urea (mM)	7.64	6.48	6.45	6.05	0.318	NBE	0.02
						BE	0.02
NH_4^+ (μM)	323	204	227	344	56.7		NS[c]
insulin[d] (ng g^{-1})	0.22 ± 0.09	0.51 ± 0.18	0.28 ± 0.14	0.55 ± 0.23		NBE	0.002
D-glucose (mM)	7.75	8.24	7.61	7.16	0.301		NS
creatinine (μM)	43.0	39.6	30.7	52.8	3.55	BE	0.02
						INT	0.002

[a] $n = 8$ for all groups except for insulin determination, for which $n = 5$, 6, 6, and 5, respectively. [b] As determined by two-way ANOVA, treating the total as a combination of the two *Y. schidigera* fractions. BE and NBE refer to the fraction determined to cause the effect of *Y. schidigera*. INT denotes significant interaction between the two fraction treatments observed in the total. [c] NS, no statistical significance observed. [d] Data presented as log-transformed data, mean ± 95% CI.

Table 3. Influence of Feed Supplementation with *Y. schidigera* Extract and Its NBE and BE Fractions on Urine Nitrogen Metabolite Concentrations[a]

variable day	control	*Y. schidigera*			SEM	effect[b]	P^b
		NBE	BE	TOT			
Urea (M)							
20	0.43	0.46	0.39	0.51	0.039		NS[c]
40	0.53	0.44	0.55	0.53	0.041		NS
60	0.57	0.59	0.58	0.61	0.035		NS
70	0.62	0.55	0.55	0.54	0.029		NS
75/76	0.60	0.67	0.54	0.52	0.051		0.08
Ammonia (mM)							
20	28.6	26.9	29.9	29.2	3.63		NS
40	24.2	15.9	20.2	23.1	2.01	INT	0.02
60	25.7	27.9	24.7	27.0	2.64		NS
70	35.8	24.4	24.1	31.6	4.93	INT	0.05
75/76	27.9	24.3	21.0	25.4	4.06		NS

[a] $n = 8$ for all groups. [b] As determined by two-way ANOVA, treating the total as a combination of the two *Y. schidigera* fractions. INT denotes significant interaction between the two fraction treatments observed in the total. [c] NS, no statistical significance observed.

Table 4. Influence of Feed Supplementation with *Y. schidigera* Extract and Its NBE and BE Fractions on Urea Cycle Enzyme Activities[a]

variable	control	*Y. schidigera*			SEM	effect[b]	P^b
		NBE	BE	TOT			
arginase (μmol min^{-1} g^{-1})	167.82	162.06	144.12	139.61	5.090	BE	0.03
ASL (μmol min^{-1} g^{-1})	3.66	3.21	3.28	2.92	0.097	BE	0.04
OTC (μmol min^{-1} g^{-1})	58.75	55.09	55.27	61.43	0.858	INT	0.002
CPS (μmol min^{-1} g^{-1})	2.28	2.49	2.51	2.48	0.059		NS[c]

[a] $n = 8$ for all groups. Results expressed on a wet weight basis. [b] As described by two-way ANOVA, treating the total as a combination of the two *Y. schidigera* fractions. BE refers to the fraction determined to cause the effect of *Y. schidigera*. INT denotes significant interaction between the two fraction treatments observed in the total. [c] NS, no statistical significance observed.

Total *Y. schidigera* extract supplementation (TOT) resulted in elevated serum creatinine concentrations (Table 2). However, analysis of the data obtained revealed that a significant decrease (29%) rather than an increase was observed in the BE group. Whereas the NBE fraction had no significant effect on its own, it appeared to interact significantly with the BE fraction to cause the increase in the recombined TOT group.

Effects on Urinary Nitrogen Variables. Urinary urea is the most important ammonia-forming product of animal wastes and constitutes ~80% of total excreted nitrogen in animals such as rats (*35*). In this study, dietary supplementation with *Y. schidigera* extract was found to have no significant effect on urinary urea concentrations on any of the sampling days. In fact, TOT-supplemented animals displayed slightly elevated urinary urea concentrations relative to control animals early in the study (Table 3, day 20). However, as the study progressed TOT-supplemented animals displayed a tendency toward decreased urinary urea concentrations, approaching significance ($P = 0.08$) at the termination of the experiment (Table 3, days 75/76). Similar observations were noted in urinary ammonia concentrations with TOT-supplemented animals having slightly increased urinary ammonia concentrations early in the study (Table 3, day 20) but lower urinary ammonia concentrations relative to controls at the termination of the experiment (Table 3, days 75/76).

Effects on Hepatic Variables. Dietary supplementation with TOT and BE significantly reduced ARG activity by 17 and 14%, respectively (Table 4). A significant decrease in ASL activity was also observed in the TOT- and BE-supplemented groups (Table 4). Although both the NBE and BE fractions decreased OTC activity, there was no overall effect in the recombined TOT (Table 4). No significant effect on CPS activity was observed in any of the treatment groups (Table 4). Overall, dietary supplementation with TOT decreased activities of ARG and ASL, with little or no change noted in CPS or OTC activities, which suggests that only the cytosolic enzymes of the urea cycle are affected by *Y. schidigera* extract.

These results show that *Y. schidigera* extract can have an effect on urea cycle enzyme activities in vivo, and this can be attributed to its BE fraction which, as already stated, contains >98% of the total sarsapogenin and smilagenin content (*23*, *24*).

However, tests in vitro indicate that *Y. schidigera* extract, at concentrations up to 1000 μg g^{-1}, had no inhibitory or stimulatory effect on the above urea cycle enzymes (Figure 1).

DISCUSSION

In this study, all three supplemented groups, TOT, NBE, and BE, displayed reduced serum urea levels (Table 2). This is consistent with previously reported observations of similar reductions in blood and serum urea concentrations in *Y. schidigera*-supplemented rats (*14*, *36*) broilers (*6*), and cattle (*16*). This could be directly related to the observed decreases in urea cycle enzyme activities, namely, ARG and ASL observed in vivo (Table 4). Although a significant decrease in urea cycle enzyme activities was observed in vivo, no direct

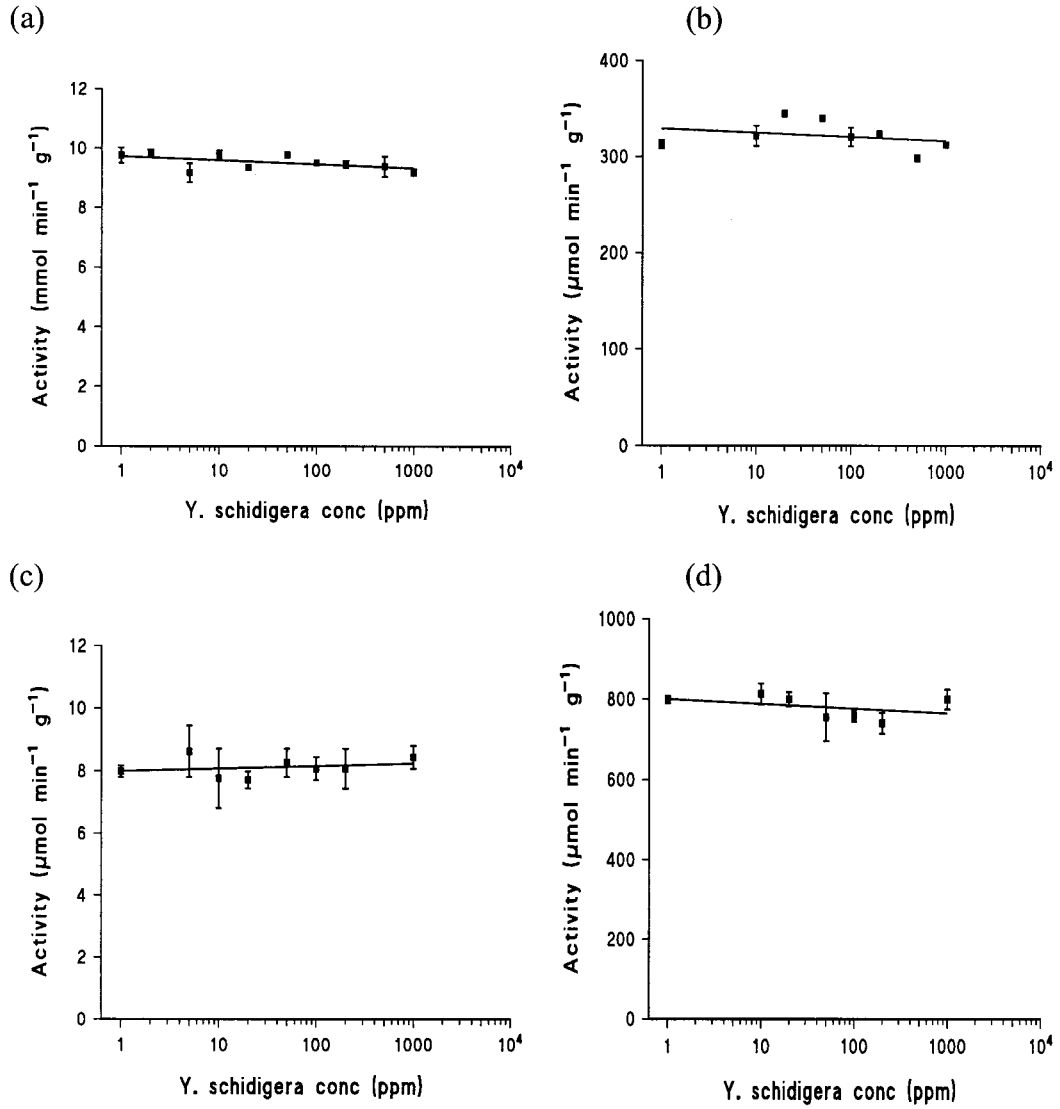

Figure 1. Effect of *Y. schidigera* extract on the activity of (a) carbamoyl phosphate synthase (CPS; EC 6.3.4.16), (b) ornithine transcarbamoylase (OTC; EC 2.1.3.3), (c) argininosuccinate lyase (ASL; EC 4.3.2.1), and (d) arginase (ARG; EC 3.5.3.1) (μmol min^{-1} g^{-1} of wet weight liver) in vitro. Error bars represent standard error of the mean (three replicates).

inhibitory or stimulatory effect was observed in liver homogenates in vitro with *Y. schidigera* extract concentrations up to 1000 μg g^{-1} (Figure 1). Therefore, the observed reductions in urea cycle enzyme activities in vivo may be due to a complex physiological process. However, the effects of normal digestive processes on the extract cannot be assessed in vitro and, thus, the possibility remains that degradation products of the extract may have an effect on urea cycle enzyme activities in vivo. Also, accumulation of the active constituents of *Y. schidigera* to effective levels over time in vivo cannot be ruled out.

Whatever the cause, these observations may be indicative of decreased protein turnover or increased protein synthesis in supplemented animals. This is supported by the observed increase in insulin concentrations in *Y. schidigera*-supplemented animals (Table 2). Insulin promotes fuel storage and protein synthesis, decreased protein degradation, and increased nitrogen balance, that is, increased muscle mass and reduced urea synthesis (*37*, *38*). Serum insulin levels were significantly increased in TOT- and NBE-supplemented groups (Table 2). The fact that serum glucose concentrations were not elevated (Table 2) indicates that the increase in serum insulin concentration is not due to an increase in serum glucose. Oser (*1*) also found no effect on blood glucose levels in rats fed *Y. schidigera* extract. Insulin can reduce the amino acid supply to the liver from tissues (*38*−*40*) and as a result can reduce the conversion of amino nitrogen to urea (*41*, *42*). This reduction in the conversion of amino nitrogen to urea could explain the observed (Table 2) and reported decreases in blood urea concentrations (*14*, *16*, *36*).

The elevated serum creatinine concentrations observed in the TOT-supplemented group (Table 2) may be interpreted in terms of increased body muscle mass in these animals. Creatinine is the sole metabolite of creatine (*43*, *44*). On a daily basis 1.5−2.0% of the total body creatine is hydrated to creatinine and excreted in the urine (*45*, *46*). Because 98% of creatine is located in striated muscle (*47*−*49*), the amount of creatinine produced by the body varies primarily with muscle mass (*50*). More recently, blood creatinine concentrations have been shown to vary linearly with muscle mass (*51*). Therefore, the elevated serum creatinine concentrations noted in the TOT-supplemented group may be interpreted in terms of increased body muscle mass in these animals. This interpretation is in agreement with the

other findings of this study, such as the decreased serum urea levels and elevated serum insulin levels, both of which may be indicative of increased nitrogen retention in the animal.

The observed effects on serum urea and hepatic urea cycle enzyme activities may have been expected to be mirrored by more dramatic effects on excreted nitrogen levels and also perhaps on growth rates. As stated previously, urinary urea is the most important nitrogen excretory formed in mammals (*35*). As a result, a more dramatic decrease in urinary urea concentrations in *Y. schidigera*-supplemented animals may have been expected. However, over the course of the experiment it should be noted that toward the termination of the study a tendency toward decreased urinary urea was noted, which approached significance on the last sampling days (Table 3), suggesting the possibility that the extract may need to accumulate to a critical level before it elicits its effects. Likewise, although no significant effect on growth rate was noted in this experiment (data not shown), there are many studies in the literature that report increased growth rate and performance (*2*, *4*, *20*).

This study indicates that the BE fraction caused the decrease in urea cycle enzyme activities, and it has previously been shown that the BE fraction contains >98% of the sarsapogenin and smilagenin content of *Y. schidigera*. Therefore, it is possible that the effect is a direct steroidal effect, perhaps even acting at a transcriptional level. However, a direct steroidal mode of action has been ruled out in the past (*2*), and the fact that the effects of BE were counteracted by the NBE in some cases (serum creatinine, Table 2) renders it necessary to be cautious about this hypothesis. In addition, the interactions noted (urinary ammonia, Table 3; OTC activity, Table 4) indicate that there may be several rather than a single active constituent, and the conflicting results with urea cycle enzyme activities in vivo versus in vitro may indicate that the active principles are actually digestive or other metabolic end products of the original plant constituents. It is also possible that the extract may not exert its effects directly on the host but rather indirectly through, for example, elements of the gastrointestinal microflora.

The influence of *Y. schidigera* extract on microbial populations has been previously reported (*17*, *52*, *53*), and an impact on gastrointestinal microfloral populations could result in decreased ammonia levels entering the bloodstream from the gastrointestinal tract, which may ultimately be reflected in a net decrease in urea synthesis in the liver.

In conclusion, therefore, although it is relatively easy to demonstrate the effects of *Y. schidigera* extract on nitrogen metabolism in animal models, more work remains to be done on elucidating the exact mode of action and the active constituents of *Y. schidigera*. Further work will have to focus on a number of issues including the possibility of the extract exerting its effects directly via a steroidal type action or indirectly via effects on the gastrointestinal microflora.

ABBREVIATIONS USED

BE, butanol extractable fraction; NBE, non-butanol extractable fraction; TOT, total *Y. schidigera* extract; ARG, arginase; ASL, argininosuccinate lyase; CPS, carbamoyl phosphate synthase; OTC, ornithine transcarbamolyase; ANOVA, analysis of variance; CI, confidence intervals; SEM, standard error of the mean; INT, interaction.

ACKNOWLEDGMENT

We thank D. Platt and B. Henderson, Department of Biological Sciences, University of Coleraine, Coleraine, Co. Derry, Northern Ireland, for performing insulin determinations.

LITERATURE CITED

(1) Oser, B. L. An evaluation of *Yucca mohavensis* as a source of food grade saponin. *Food Cosmet. Toxicol.* **1966**, *4*, 57−61.

(2) Hale, W. H.; Sherman, W. C.; Reynolds, W. M.; Luther, H. G.; English, A. R. The value of certain steroidal sapogenins in rations of fattening lambs and cattle. *Proc. Soc. Exp. Biol. Med.* **1961**, *105*, 486-489.

(3) Johnson, N. L.; Quarles, C. L.; Fagerberg, D. J.; Cavens, D. D. Evaluation of *Yucca* saponin on broiler performance and ammonia suppression. *Poult. Sci.* **1981**, *60*, 2289−2292.

(4) Anthony, N. B.; Balog, J. M.; Staudinger, F. B.; Wall, C. W.; Walker, R. D.; Huff, W. E. Effect of a urease inhibitor and ceiling fans on ascites in broilers. 1. Environmental variability and incidence of ascites. *Poult. Sci.* **1994**, *73*, 801−809.

(5) Goodall, S. R.; Eichenblaum, J. D.; Matsushima, J. K. Sarsaponin and monensin effects upon *in vitro* VFA concentration, gas production and feedlot performance. *J. Anim. Sci.* **1979**, *49* (Suppl.), 370−371.

(6) Balog, J. M.; Anthony, N. B.; Wall, C. W.; Walker, R. D.; Rath, N. C.; Huff, W. E. Effect of an urease inhibitor and ceiling fans on ascites in broilers. 2. Blood variables, ascites scores and body and organ weights. *Poult. Sci.* **1994**, *73*, 810−816.

(7) Cline, J. L.; Fisher, B. A.; Trottier, N. L.; Walker, R. D.; Easter, R. A. Effect of feeding Micro-Aid on stillbirths, pre-weaning mortality, blood oxygen values of piglets and blood urea nitrogen in sows. *J. Anim. Sci.* **1996**, *74* (Suppl.), 189.

(8) Rowland, L. O.; Plyler, J. E.; Bradley, J. W. *Yucca schidigera* extract effect on egg production and house ammonia levels. *Poult. Sci.* **1976**, *55*, 2086.

(9) Ellenberger, M. A.; Rumpler, W. V.; Johnson, D. E.; Goodall, S. R. Evaluation of the extent of ruminal urease inhibition by sarsaponin and sarsaponin fractions. *J. Anim. Sci.* **1985**, *61* (Suppl.), 491−492.

(10) Headon, D. R.; Buggle, K. A.; Nelson, A. B.; Killeen, G. F. Glycofractions of the *Yucca* plant and their role in ammonia control. In *Biotechnology in the Feed Industry: Proceedings of Alltech's Seventh Annual Symposium*; Lyons, T. P., Ed.; Alltech Technical Publications: Nicholasville, KY, 1991; pp 95−108.

(11) Aspund, R. O. Urease inhibition by extracts and extract fractions from species of the plant genus *Yucca*. *J. Anim. Sci.* **1991**, *69* (Suppl. 1), 113.

(12) Sutton, A. L.; Goodall, S. R.; Patterson, J. A.; Mathew, A. G.; Kelly, D. T.; Meyerholtz, K. A. Effects of odour control compounds on urease activity in swine manure. *J. Anim. Sci.* **1992**, *70* (Suppl. 1), 160.

(13) Killeen, G. F.; Buggle, K. A.; Hynes, M. J.; Walsh, G. A.; Power, R. F.; Headon, D. R. Influence of *Yucca schidigera* preparations on the activity of urease from *Bacillus pasteurii*. *J. Sci. Food Agric.* **1994**, *65*, 433−440.

(14) Killeen, G. F.; Connolly, C. R.; Walsh, G. A.; Duffy, C. F.; Headon, D. R.; Power, R. F. The effects of dietary supplementation with *Yucca schidigera* extract or fractions thereof on nitrogen metabolism and gastrointestinal fermentation processes in the rat. *J. Sci. Food Agric.* **1998**, *76*, 91−99.

(15) Peestock, L. A. An investigation into the application of *Yucca schidigera* extracts to biological waste treatments. M.Sc. Thesis, Miami University, Oxford, OH, 1979.

(16) Hussain, I.; Cheeke, P. R. Effect of dietary *Yucca schidigera* extract on rumen and blood profiles of steers fed concentrate- or roughage-based diets. *Anim. Feed Sci. Technol.* **1995**, *51*, 231−242.
(17) Wallace, R. J.; Arthaud, L.; Newbold, C. J. Influence of *Yucca schidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. *Appl. Environ. Microbiol.* **1994**, *60*, 1762−1767.
(18) Gibson, M. L.; Preston, R. L.; Pritchard, R. H.; Goodall, S. R. Effect of Sarsaponin and monensin on rumen ammonia levels and *in vitro* dry-matter digestibilities. *J. Anim. Sci.* **1985**, *61* (Suppl.), 492−495.
(19) van Nevel, C. J.; Demeyer, D. I. Effect of antibiotics, a deaminase inhibitor and sarsaponin on nitrogen metabolism of rumen contents *in vitro*. *Anim. Feed Sci. Technol.* **1990**, *31*, 323−348.
(20) Goodall, S. R.; Matsushima, J. K. Sarsaponin and monensin effects ruminal VFA concentrations and weight gains of feedlot cattle. *J. Anim. Sci.* **1979**, *49* (Suppl.), 371−372.
(21) Price, K. R.; Johnson, I. T.; Fenwick, G. R. The chemistry and biological significance of saponins in foods and feedingstuffs. *CRC Crit. Rev. Food Sci. Nutr.* **1987**, *6*, 27−135.
(22) Kaneda, N.; Nakanishi, H.; Staba E. J. Steroidal constituents of *Yucca schidigera* plants and tissue cultures. *Phytochemistry* **1987**, *26*, 1425−1429.
(23) Connolly, C. R. Quantitative analysis of 3-β-hydroxy-5-β-spirostane sterols and their glycosides in *Yucca schidigera* extracts and supplemented feed. M.Sc. Thesis, Department of Biochemistry, National University of Ireland, Galway, Ireland, 1995.
(24) Killeen, G. F.; Madigan, C. A.; Connolly, C. R.; Walsh, G. A.; Clark, C.; Hynes, M. J.; Timmins, B. F.; James, P.; Headon, D. R.; Power, R. F. Antimicrobial saponins of *Yucca schidigera* and the implications of their *in vitro* properties for their *in vivo* impact. *J. Agric. Food Chem.* **1998**, *46*, 3178−3186.
(25) Hardeland, R. Species differences in the diurnal rhythmicity of courtship behavior within the melanogaster group of the genus *Drosophila*. *Anim. Behav.* **1972**, *20*, 170−174.
(26) Fuentes, J. M.; Paschal, M. R.; Salido, G.; Soler, G. Oscillations in rat liver cytosolic enzyme activities in the urea cycle. *Arch. Int. Phys. Biochim. Biophys.* **1994**, *102*, 237−241.
(27) Mondzac, A.; Ehrlich, G. E.; Seegmiller, J. E. An enzymatic determination of ammonia in biological fluids. *J. Lab. Clin. Med.* **1965**, *66*, 526−531.
(28) Chaney, A. L.; Marbach, E. P. Modified reagents for the determination of urea and ammonia. *Clin. Chem.* **1962**, *8*, 130−132.
(29) Heding, L. Determination of insulin in insulin treated diabetic patients. *Diabetologia* **1972**, *8*, 260−268.
(30) Schimke, R. T. Differential effects of fasting and protein free diets on levels of urea cycle enzymes in rat liver. *J. Biol. Chem.* **1962**, *237*, 459−468.
(31) Nazum, I.; Snodgrass, P. J. Multiple assays of the five urea cycle enzymes in human liver homogenates. In *The Urea Cycle*; Grisolia, S., Bagmena, R., Mayor, F., Eds.; Wiley: New York, 1976; pp 325−349.
(32) Sokal, R. R.; Rohlf, F. J. *Biometry*, 2nd ed.; Freeman: San Francisco, CA, 1981.
(33) Miller, J. C.; Miller, J. N. *Statistics for Analytical Chemistry*; Wiley: Chichester, U.K., 1988.
(34) Murray, A. W. A. Recommendations of the editorial board on use of statistics in papers submitted to JSFA. Guidelines to authors. *J. Sci. Food Agric.* **1988**, *42*, v−ix.
(35) Estornell, E.; Barber, T.; Cabo, J. Improved nitrogen-metabolism in rats fed on lipid-rich liquid diets. *Br. J. Nutr.* **1994**, *71*, 361−373.
(36) Preston, R. L.; Bartle, S. J.; May, T.; Goodall, S. R. Influence of sarsaponin on growth feed and nitrogen utilisation in growing male rats fed diets with added urea or protein. *J. Anim. Sci.* **1987**, *65*, 481−487.
(37) Millward, D. J. The physiological regulation of proteolysis in muscle. *Biochem. Soc. Trans.* **1985**, *13*, 1023−1026.
(38) Meijer, A. J.; Lamers, W. H.; Chamuleau, R. A. F. M. Nitrogen metabolism and ornithine cycle function. *Physiol. Rev.* **1990**, *70*, 701−749.
(39) Pozefsty, T.; Felig, P.; Tobin, J. D.; Soeldner, J. S.; Cahill, G. R. J. Amino acid balance across tissues of the forearm in postabsorptive man. Effects of insulin at two dose levels. *J. Clin. Invest.* **1969**, *48*, 2273−2282.
(40) Moldawer, L. L.; Swaninger, G.; Gelin, J.; Lundholm, K. Interleukin-1 and tumour necrosis factor do not regulate protein balance in skeletal muscle. *Am. J. Physiol.* **1987**, *253*, C766−C773.
(41) Vilstrup, H. Effects of glucose on alanine-derived urea synthesis. *Clin. Physiol.* **1984**, *4*, 495−507.
(42) Jahoor, F.; Wolfe, R. R. Regulation of protein catabolism. *Kidney Int. Suppl.* **1987**, *22*, 81−93.
(43) Munro, H. N.; Crim, M. C. The proteins and amino acids. In *Modern Nutrition in Health and Disease*; Shils, M. E., Young, V. R., Eds.; Lea and Febiger: Philadelphia, PA, 1988; pp pp 1−37.
(44) Finco, D. R. Kidney function. In *Clinical Biochemistry of Domestic Animals*; Kaneko, J. J., Ed.; Academic Press: San Diego, CA, 1989; pp 486−542.
(45) Rodwell, V. W. Conversion of amino acids to specialized products. In *Review of Physiological Chemistry*; Harper, H. A., Rodwell, V. W., Mayes, P. A., Eds.; Lange Medical Publications: Los Altos, CA, 1979; pp 430−439.
(46) DelGiudice, G. D.; Asleson, M. A.; Varner, L. W.; Hellgren, E. C. Twenty-four-hour urinary creatinine and urea nitrogen excretion in male white-tailed deer. *Can. J. Zool.* **1995**, *73*, 493−501.
(47) Meador, C. K.; Kreisberg, R. A.; Friday, J. P.; Bowdoin, B.; Coan, P.; Armstrong, J.; Hazelrig, J. B. Muscle mass determination by isotopic dilution of creatine-^{14}C. *Metab. Clin. Exp.* **1968**, *17*, 1104−1108.
(48) Waterlow, J. C.; Neale, R. J.; Rowe, L.; Palin, I. Effects of diet and infection on creatine turnover in the rat. *Am. J. Clin. Nutr.* **1992**, *25*, 371−375.
(49) Demant, T. W.; Rhodes, E. C. Effects of creatine supplementation on exercise performance. *Sport Med.* **1999**, *28*, 49−60.
(50) Cheeke, D. B.; Brasel, J. A.; Elliott, D.; Scott, R. Muscle cell size and number in normal children and in dwarfs (pituitary cretins and primordial) before and after treatment. *Bull. Johns Hopkins Hosp.* **1966**, *119*, 46−62.
(51) Schutte, J. E.; Longhurst, J. C.; Gaffney, F. A.; Bastien, B. C.; Blomquist, C. G. Total plasma creatinine: An accurate measure of total striated muscle mass. *J. Appl. Physiol.* **1981**, *51*, 762−766.
(52) Sen, S.; Makkar, H. P. S.; Muetzel, S.; Becker, K. Effect of *Quillaja saponaria* saponins and *Yucca schidigera* plant extract on growth of *Escherichia coli*. *Lett. Appl. Microbiol.* **1998**, *27*, 35−38.
(53) Wang, Y.; McAllister, T. A.; Yanke, L. J.; Cheeke, P. R. Effect of steroidal saponin from *Yucca schidigera* extract on ruminal microbes. *J. Appl. Microbiol.* **2000**, *88*, 887−896.

Received for review February 5, 2001. Revised manuscript received April 27, 2001. Accepted April 27, 2001.

JF010149+